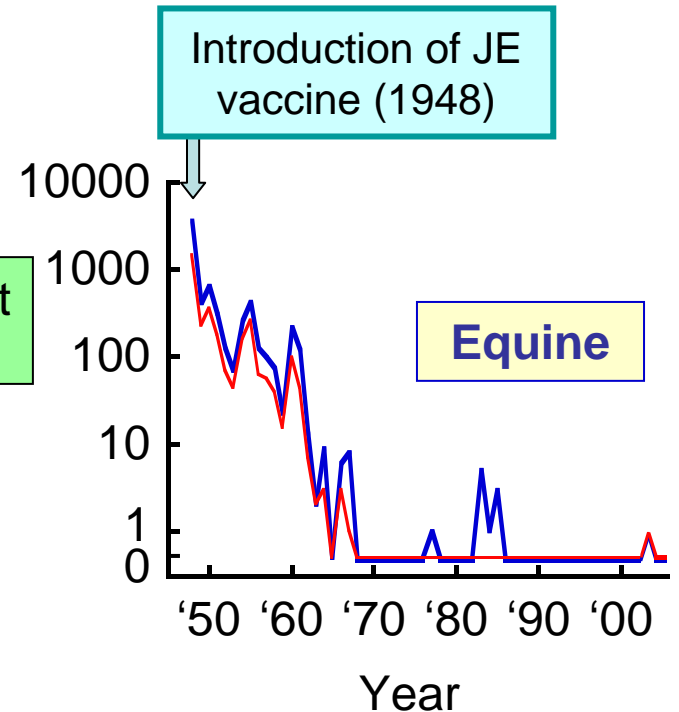
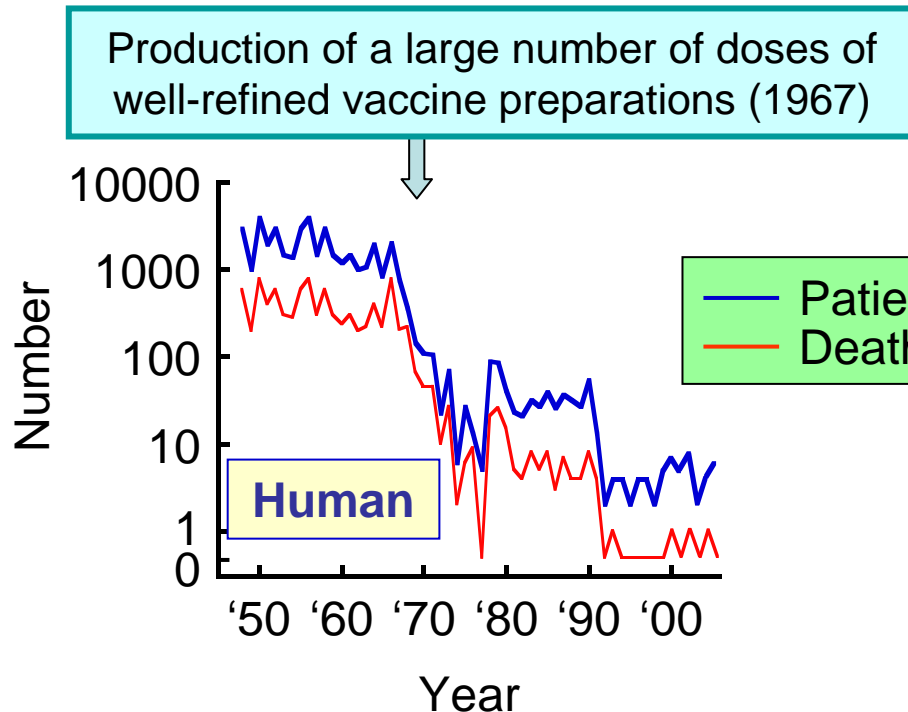
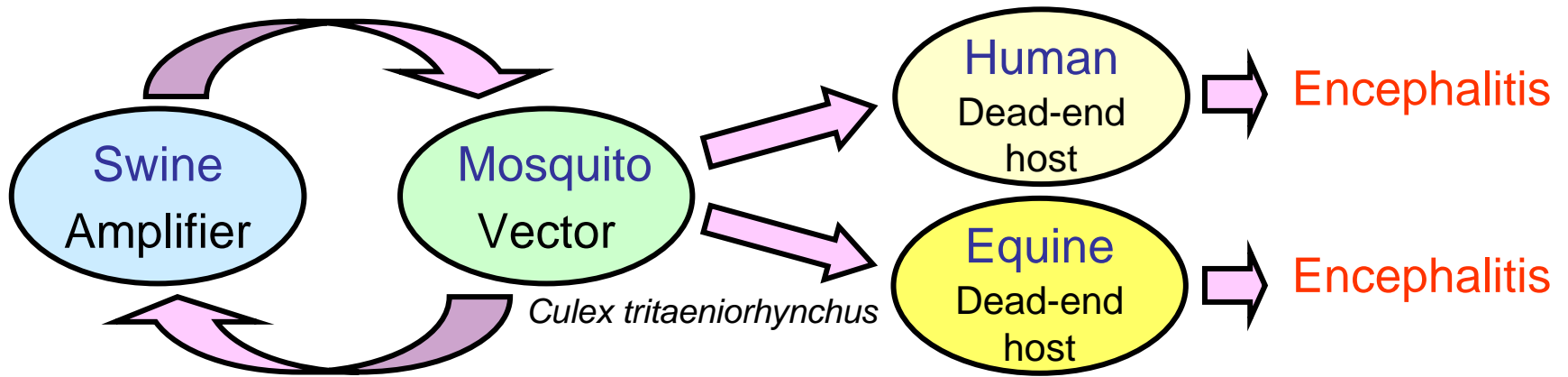


## Continued transmission and need for booster doses in an endemic country

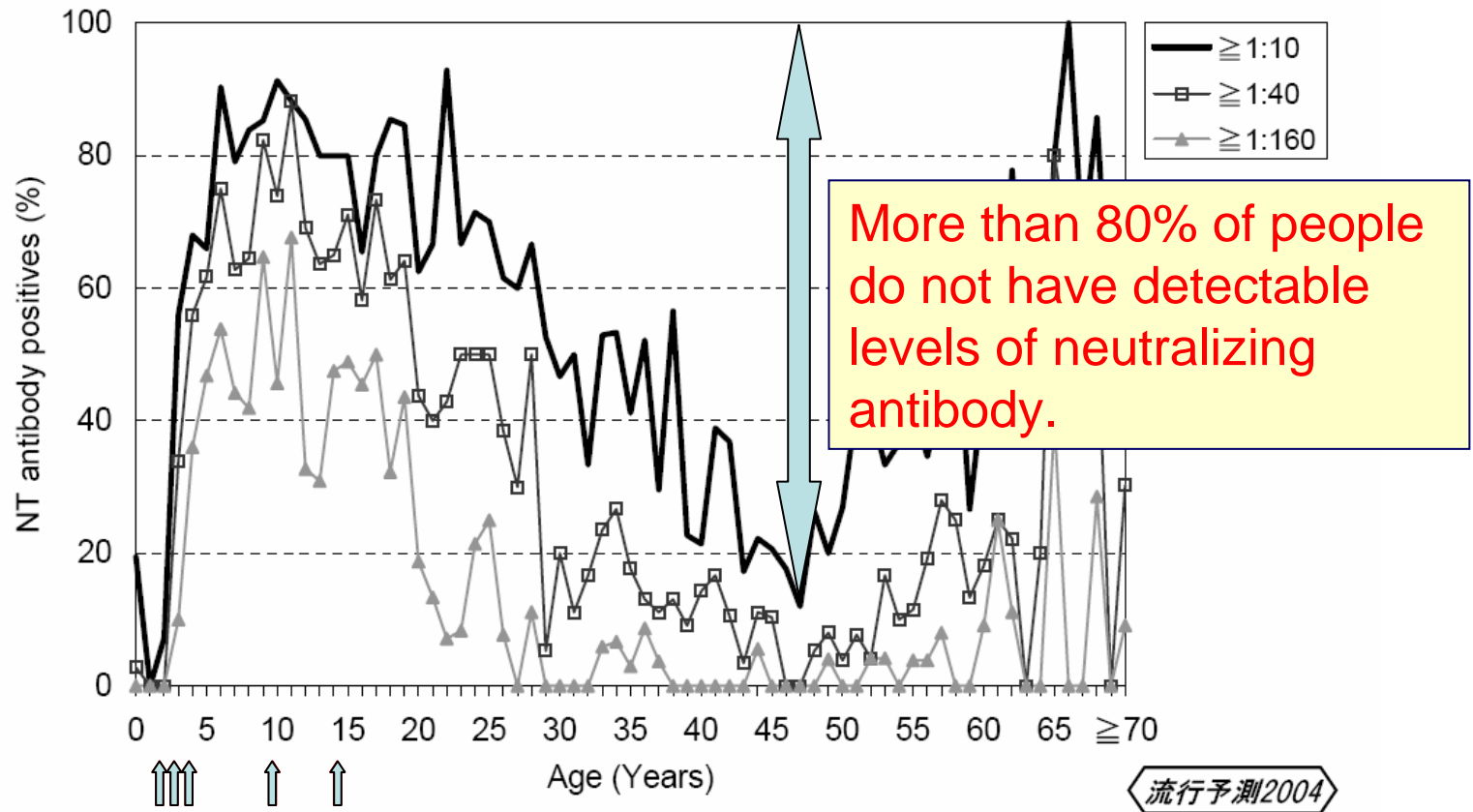
Eiji Konishi\*, Mizue Shoda\*, Tomoyuki Suzuki\*, Takashi Kondo\*\*,  
Satoru Arai\*\*\*, Keiko Taya\*\*\*, Nobuhiko Okabe\*\*\*

Kobe University\*, Japan Racing Association\*\*,  
National Institute of Infectious Diseases\*\*\*

# Life Cycle of Japanese encephalitis virus



# Age distribution of Japanese encephalitis neutralizing antibody positives, 2004

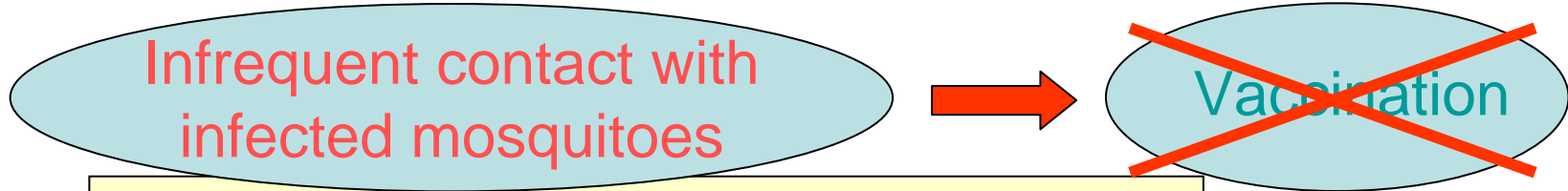


from National Institute of Infectious Diseases (NIID), Japan

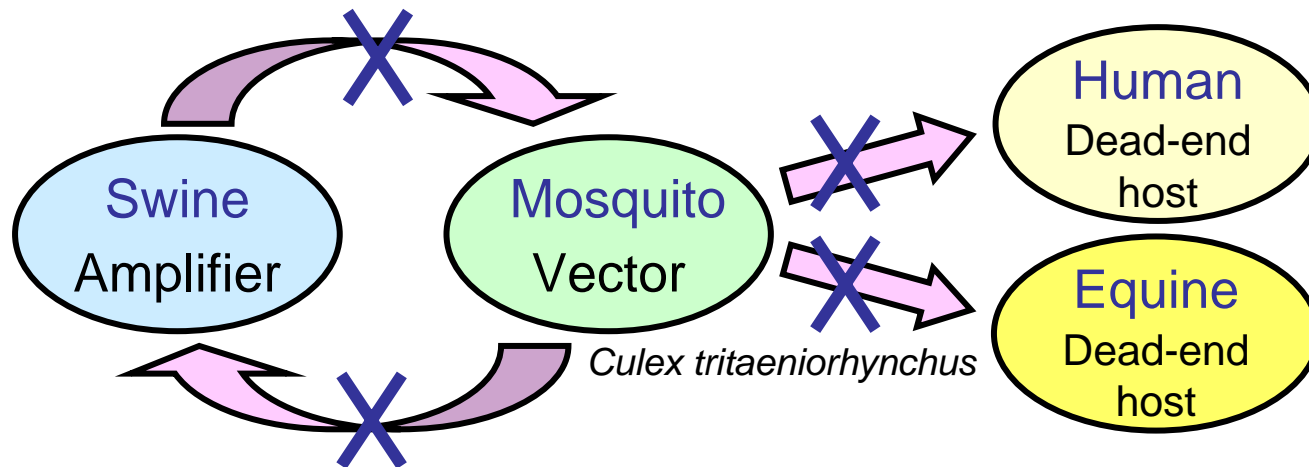
Why were these people protected from JE?

Antibody-mediated immunity after 45 years of age

Diseases by Infection Disease Surveillance Center, NIID, Japan



Use of insecticides  
Improved irrigation schemes for rice cultivation  
Relocation of many pig farms



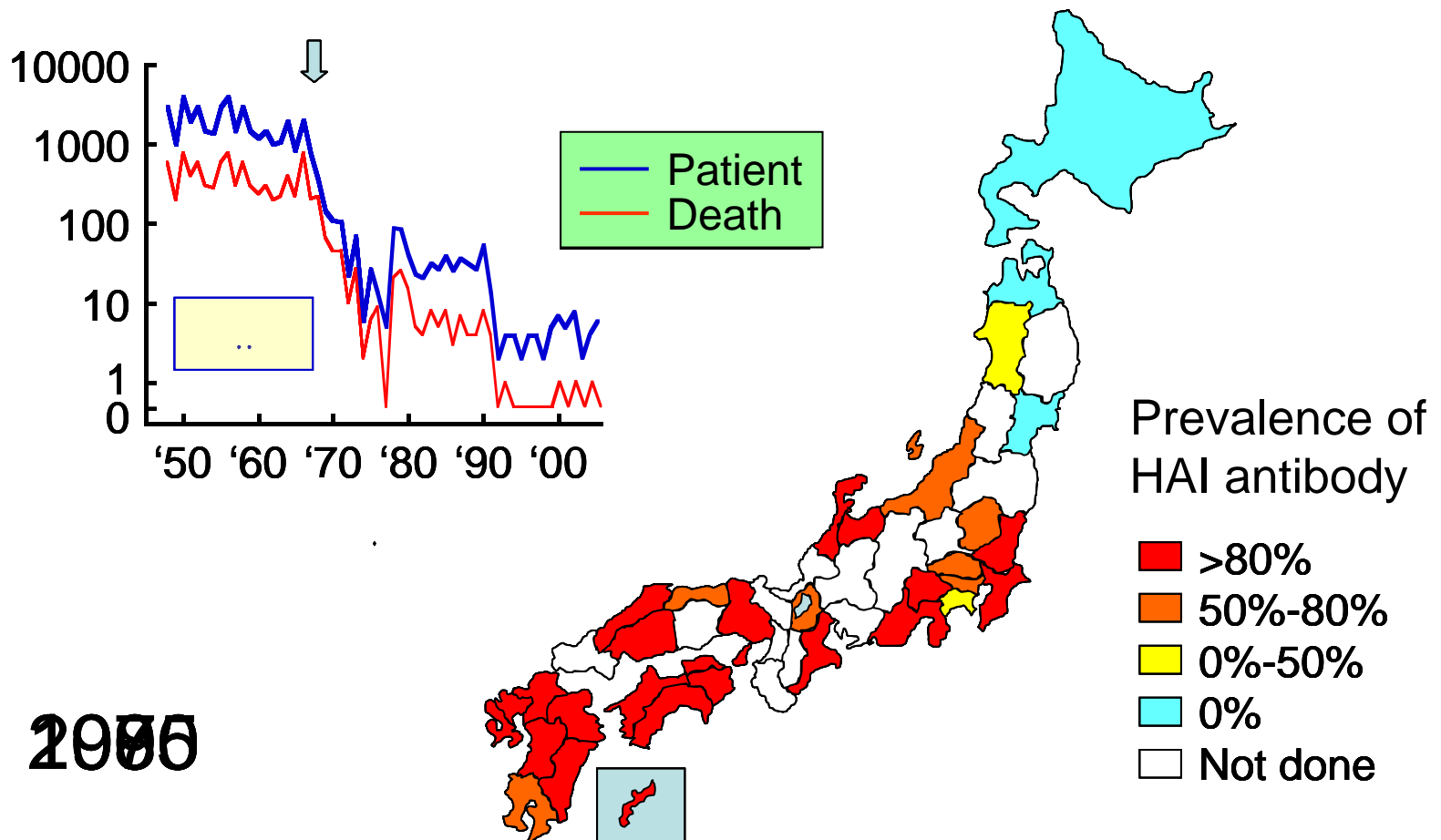
Natural JEV activity?

# Continued transmission and need for booster doses in an endemic country

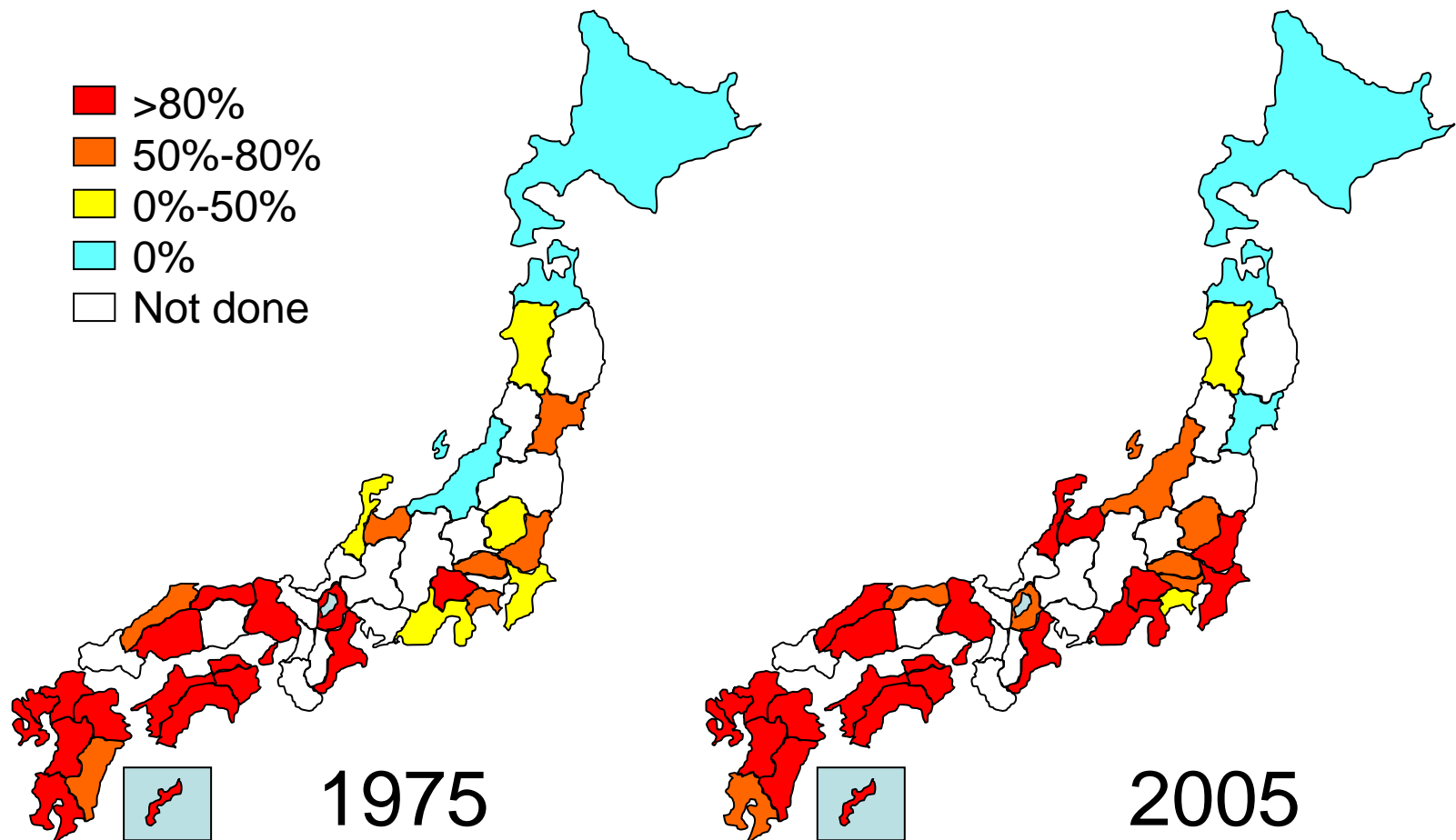
1. Natural JEV activity in Japan
2. Development of a method detecting natural infections
3. Nationwide survey among human populations
4. Anamnestic response and “natural” boosting

# Surveillance of swine infected by JEV

**National Epidemiological Surveillance of Vaccine-preventable Diseases** by Infectious Disease Surveillance Center, NIID, Japan

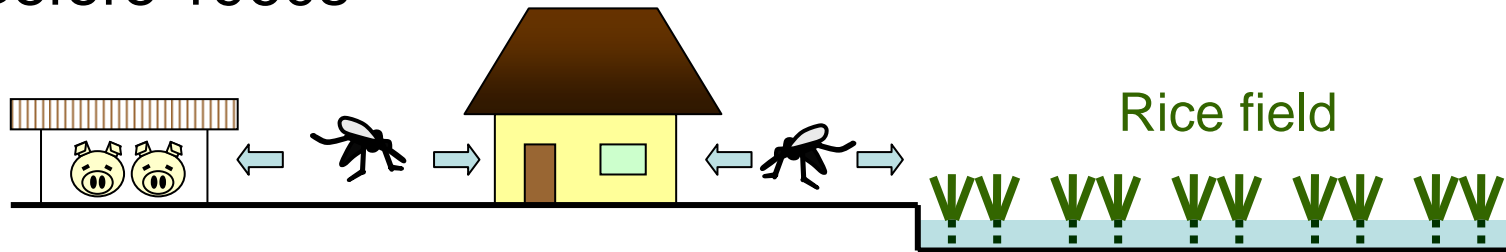


# JEV is still circulating in Japan

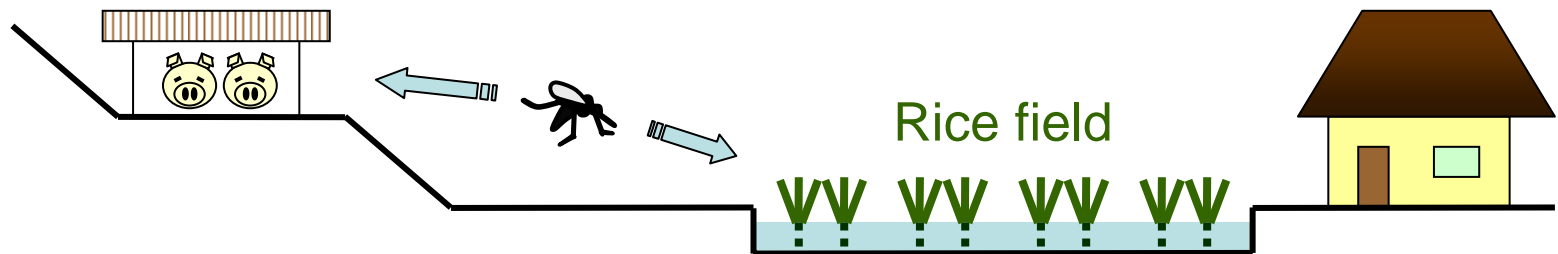


# Relocation of pig farms

Before 1960s



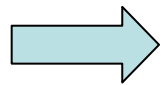
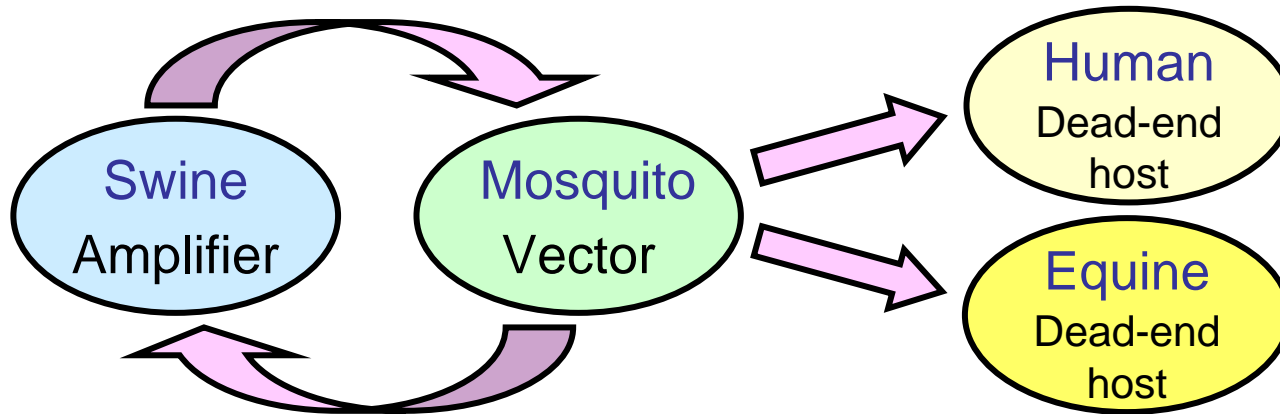
Recent years



Prevalence of antibody among swine cannot always be used for estimating natural infection rates in humans.



## Life Cycle of Japanese encephalitis virus



Survey of natural JEV infections among humans is more straightforward.

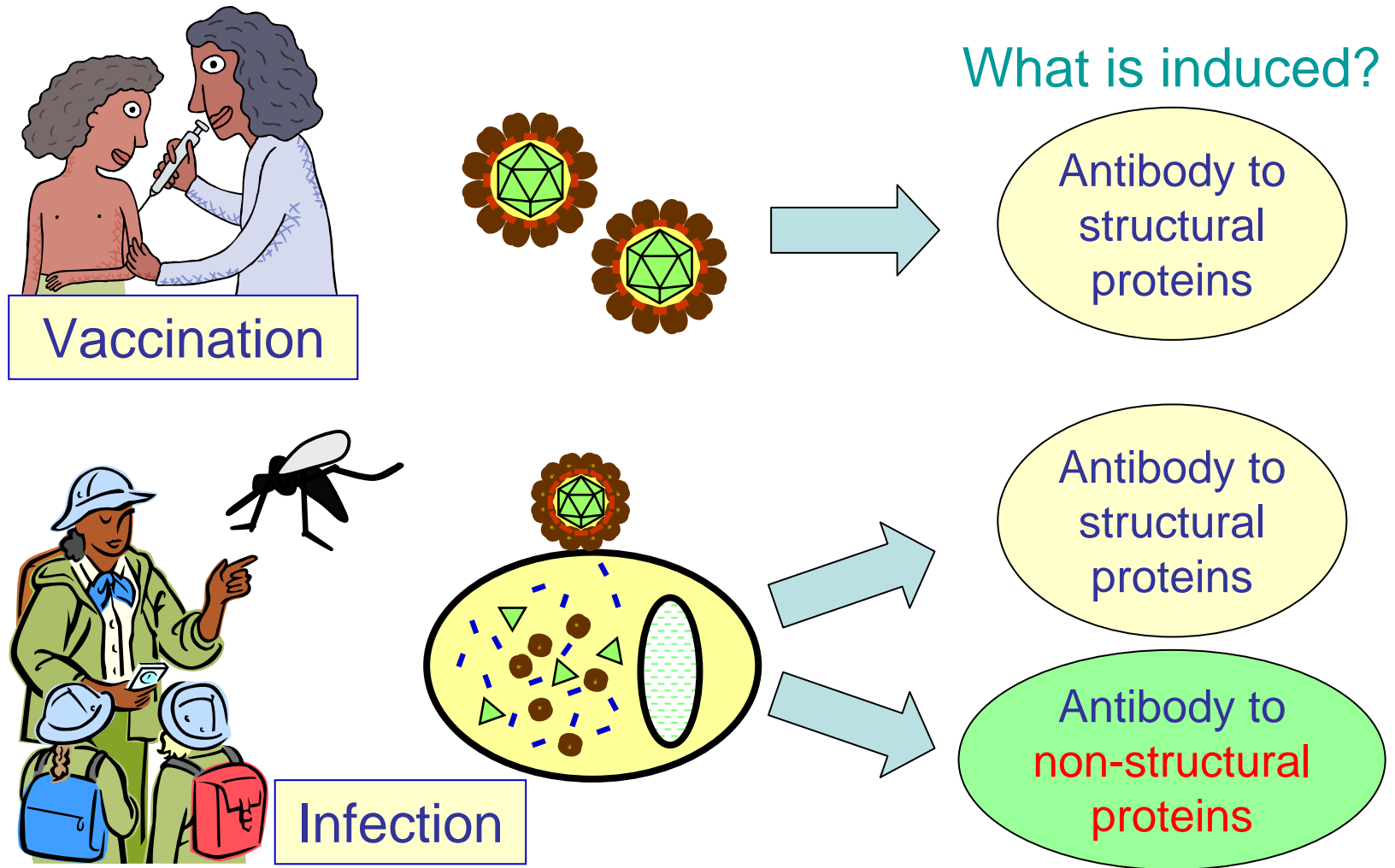
Neutralization and HAI tests cannot be used to obtain natural infection rates among vaccinated populations, since these methods do not differentiate antibodies induced by natural infection from those induced by vaccination.



# Continued transmission and need for booster doses in an endemic country

1. Natural JEV activity in Japan
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# Principle of the method detecting natural infections

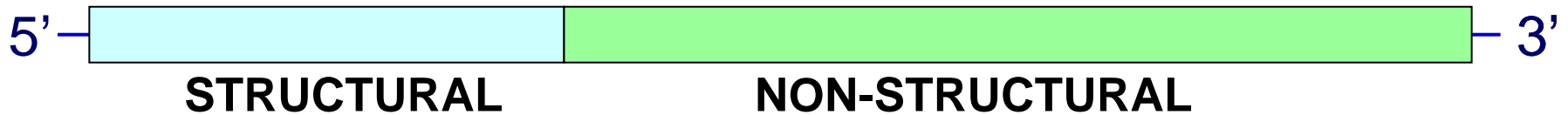


➔ Detection of antibodies to non-structural proteins can differentiate infected from uninfected individuals in vaccinated populations

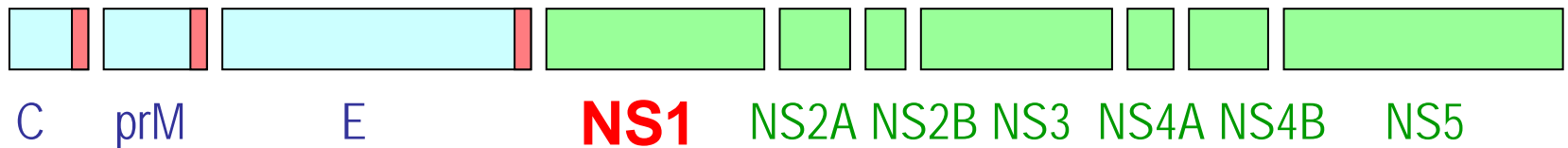
NS1 would be the best antigen used in an antibody detection system

## JEV GENOME

10,300 base OPEN READING FRAME



### MAJOR PROTEOLYTIC PRODUCTS



Secreted from  
infected cells



Antibodies to NS1 can be  
induced effectively in the host.

# Detection of NS1 antibody by an immunostaining method

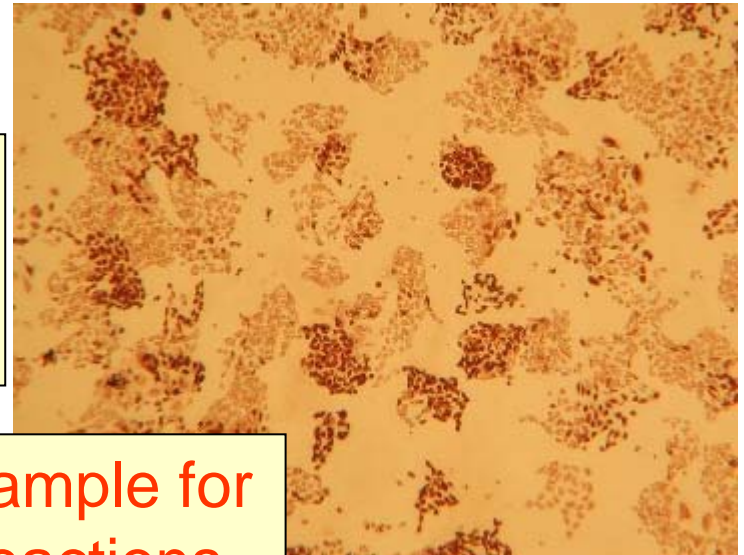
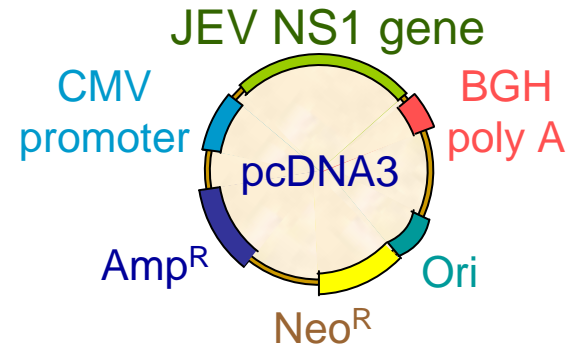
Construct a plasmid encoding the NS1 gene



Generate a CHO cell line stably expressing NS1

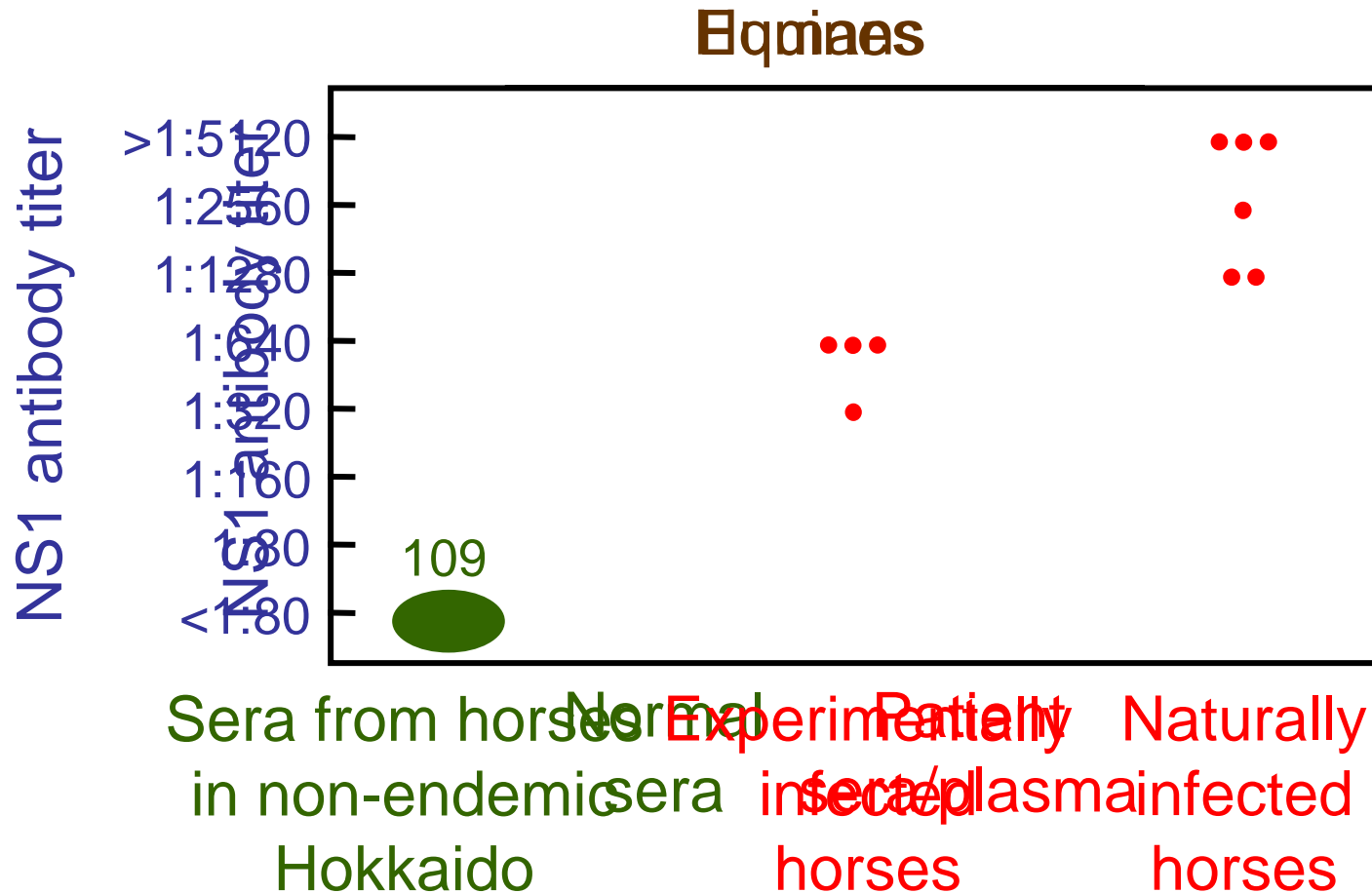


Develop a method to measure NS1 antibody based on immunostaining



Typical example for positive reactions

# Comparison of NS1 antibody titers



NS1 antibodies can constitute a marker of natural infection.

# Continued transmission and need for booster doses in an endemic country

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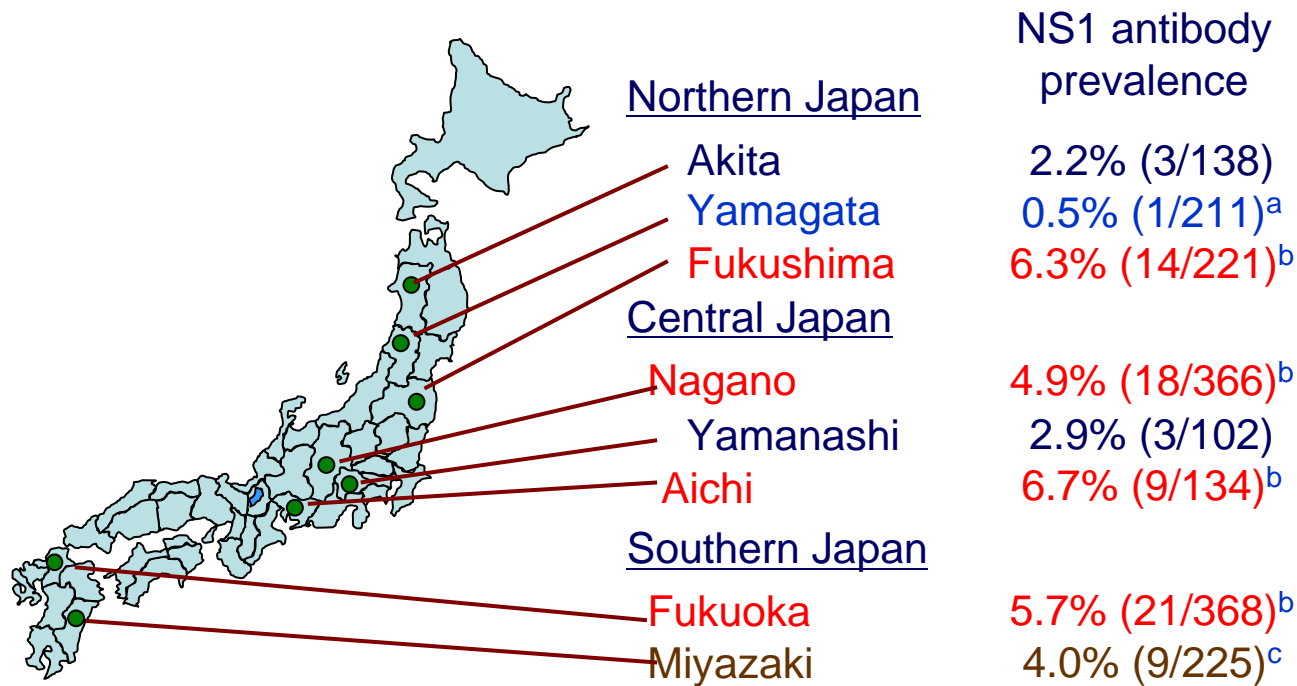
# Human sera provided by “Serum Bank” of NIID

Collected in 2001

Area	Location	Number of samples		
		Male	Female	Total
Northern	Akita	103	35	138
	Yamagata	100	111	211
	Fukushima	135	86	221
Central	Nagano	195	171	366
	Yamanashi	33	69	102
	Aichi	89	45	134
Southern	Fukuoka	168	200	368
	Miyazaki	97	128	225
Total		920	845	1765



# Prevalence of NS1 antibody among human populations in 8 prefectures of Japan, 2001



<sup>a-b</sup> $P < 0.01$ , <sup>a-c</sup> $P < 0.05$

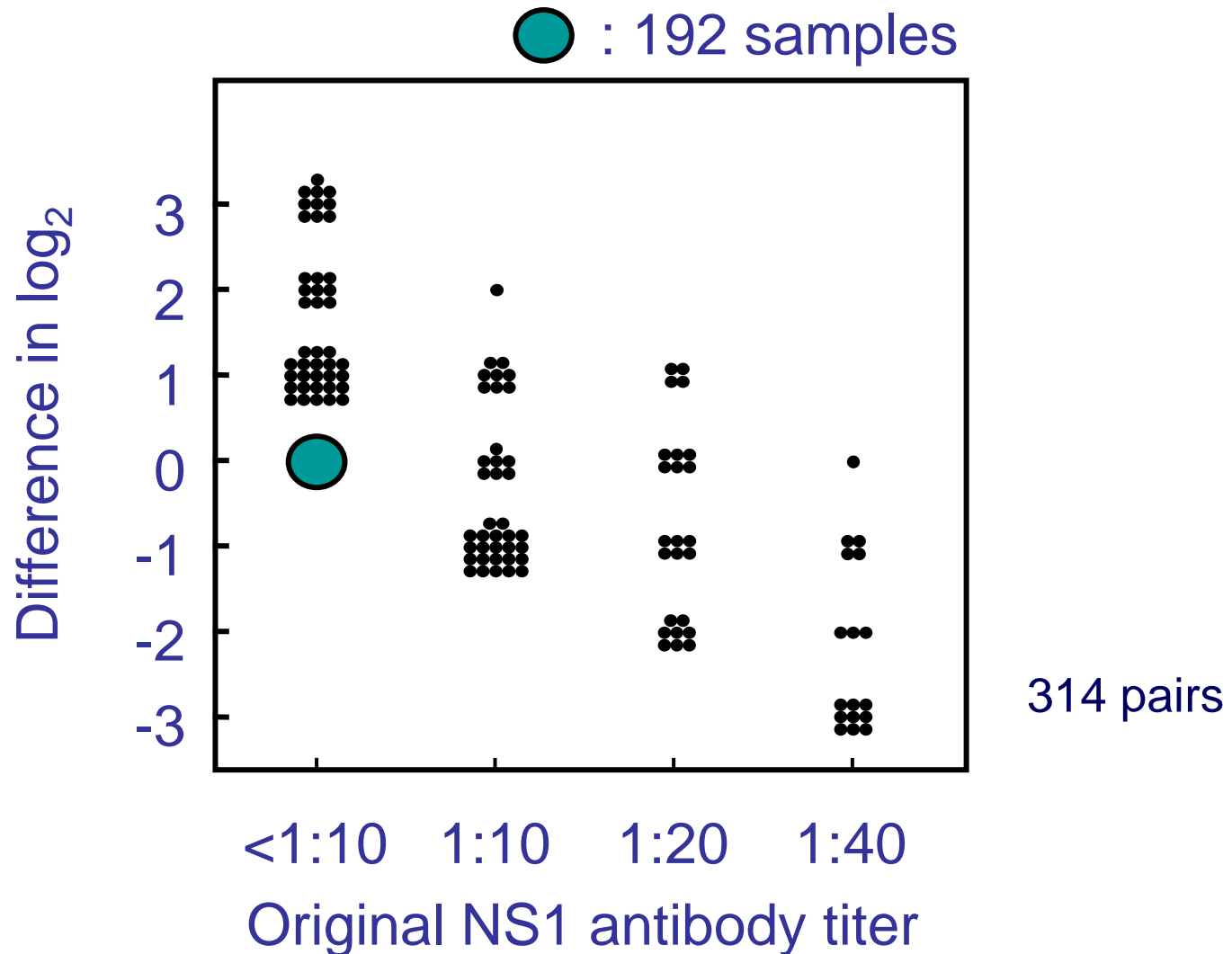
Antibody prevalence at one time point

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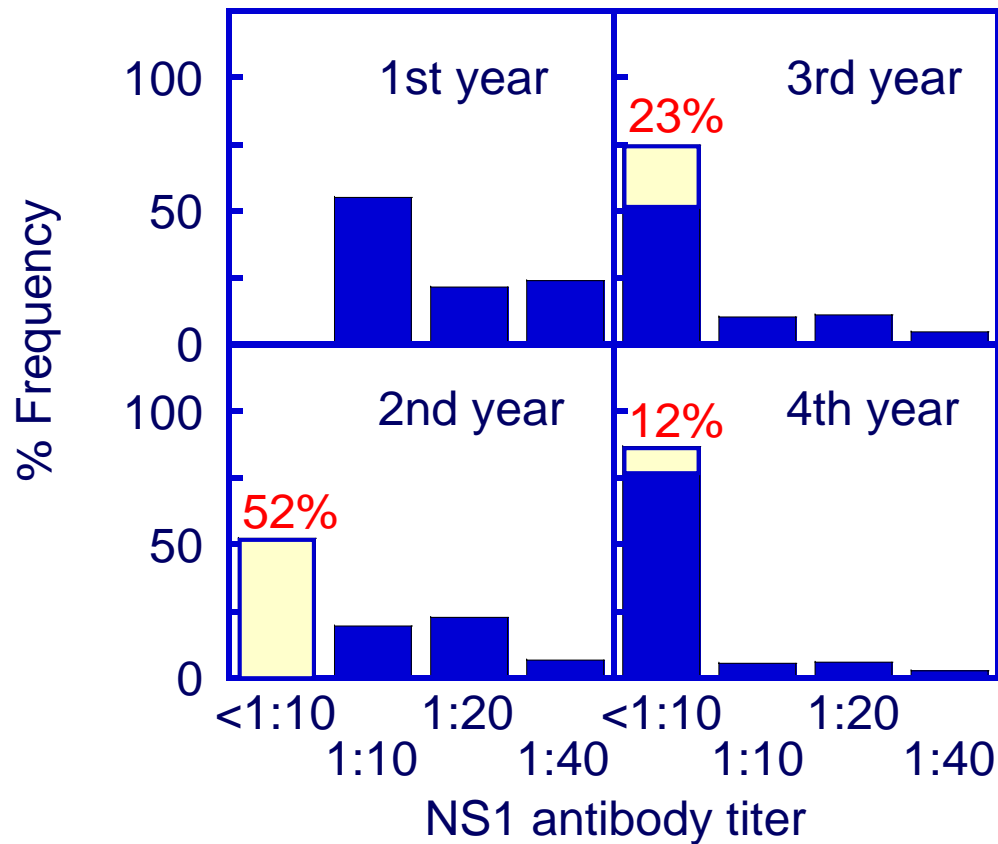
Duration of antibody response

◊ Annual infection rate

# Yearly change in NS1 antibody titer



# Simulation of yearly changes in frequency distributions of NS1 antibody titers and in yearly seroreversion rates



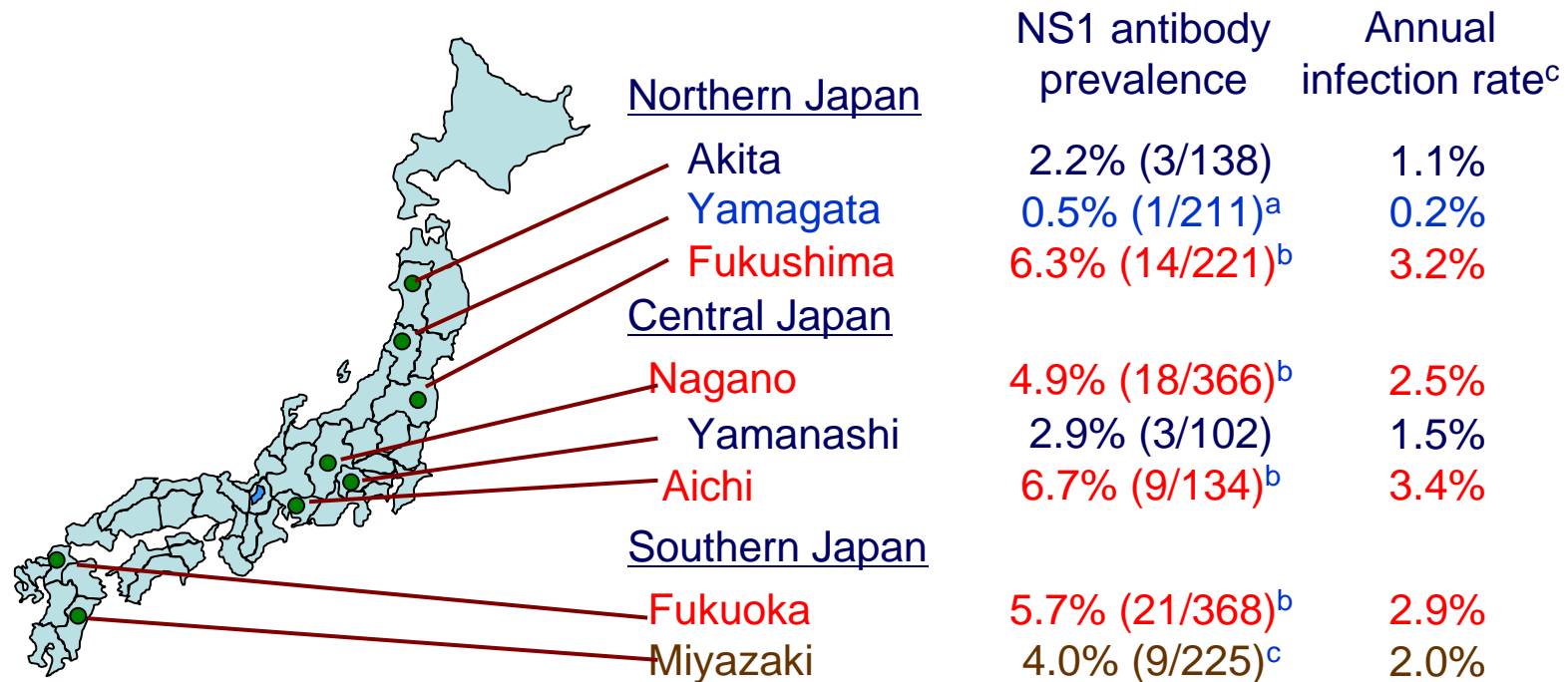
## Calculation of the mean period between seroconversion and seroreversion

Years to seroreversion (A)	% Seroreverting Population (B)	Cumulative % of negative population	A x B
.	52.02	52.02	52.02
2	22.59	74.61	45.18
3	12.09	86.70	36.27
4	6.32	93.02	25.29
5	3.32	96.34	16.59
6	1.74	98.08	10.44
7	0.91	98.99	6.39
8	0.48	99.47	3.83
9	0.25	99.72	2.26
10	0.13	99.85	1.32
11	0.07	99.92	0.76
12	0.04	99.96	0.43

Mean duration of NS1 antibody response:  $200.77 / 99.96 = 2.01$  years

# Prevalence of NS1 antibody among human populations in 8 prefectures of Japan, 2001

(sera provided by “Serum Bank” of NIID)



<sup>a-b</sup> $P < 0.01$ , <sup>a-c</sup> $P < 0.05$

<sup>c</sup>Based on the duration of NS1 antibody responses of 2 years

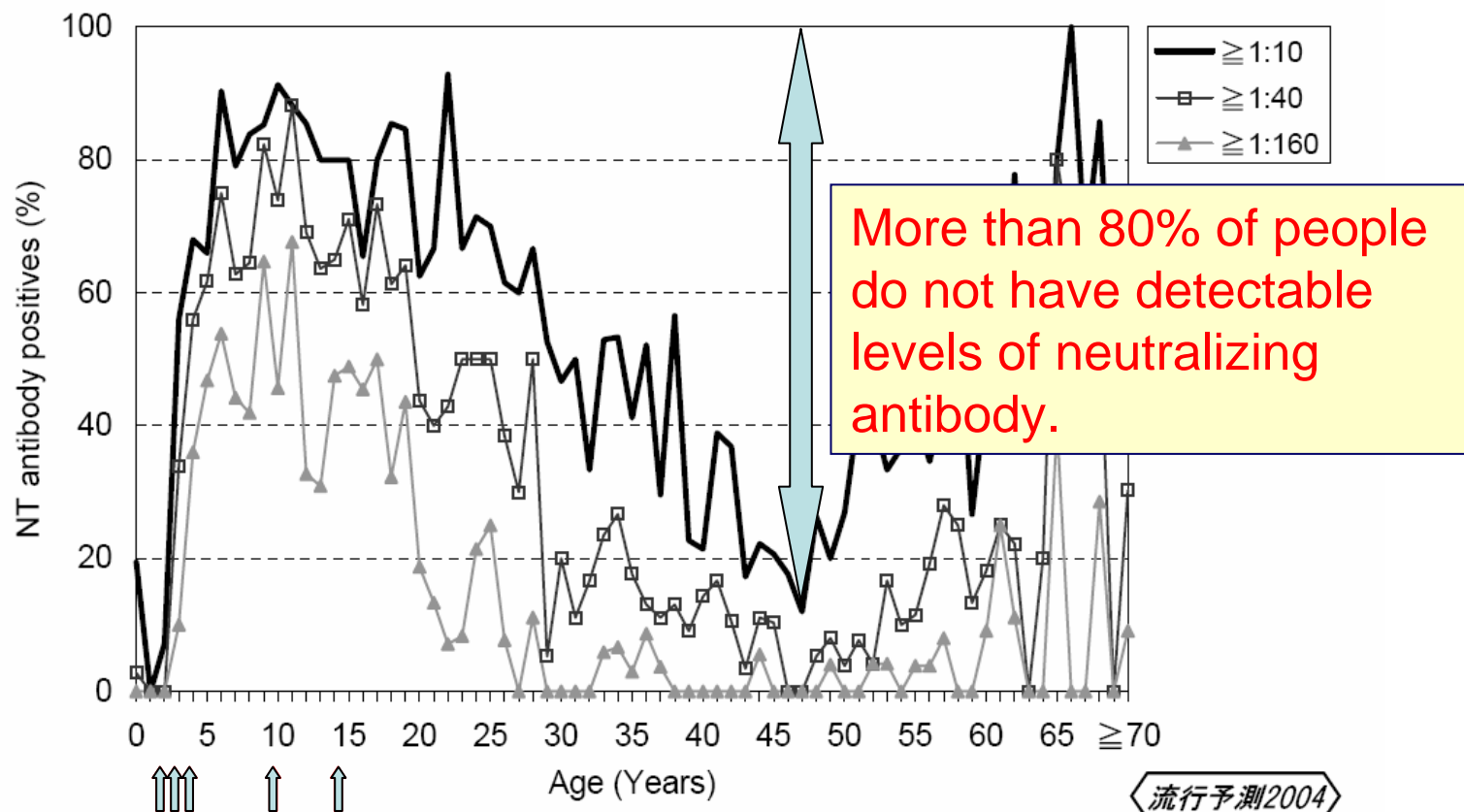


Human populations in Japan are still exposed to natural JEV infections.



Consistent with swine data

## Age distribution of Japanese encephalitis neutralizing antibody positives, 2004



Why were these people protected from JE?

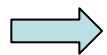
# Continued transmission and need for booster doses in an endemic country

1. Natural JEV activity in Japan
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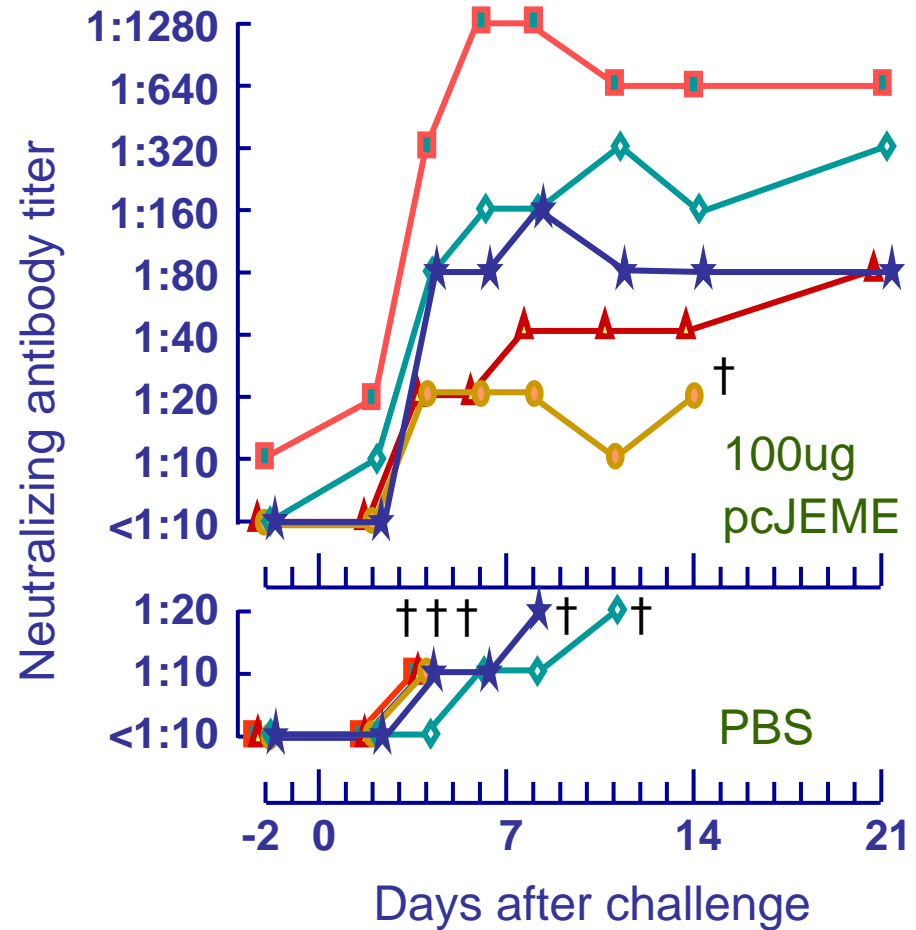
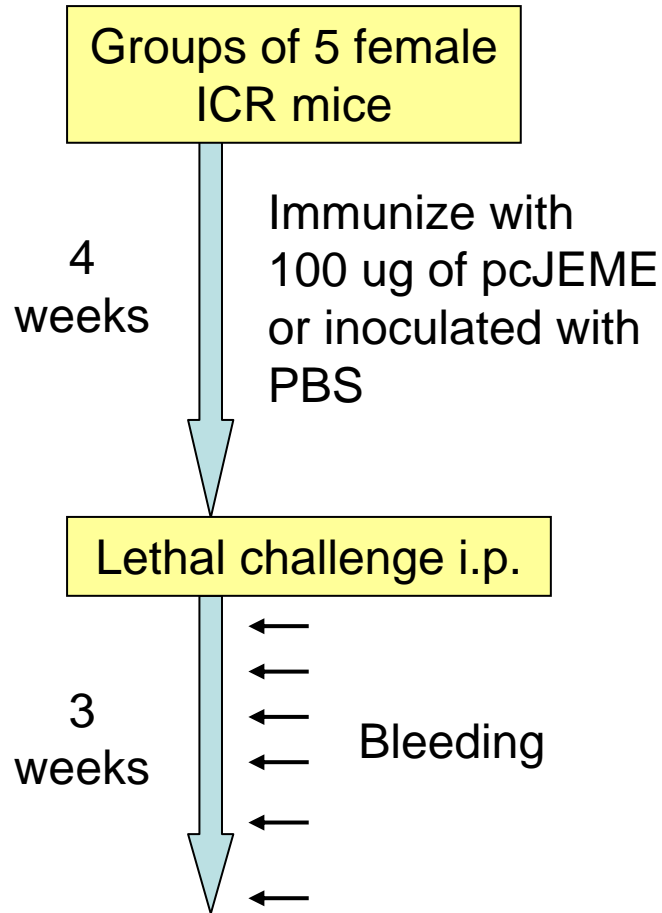
Immunization of mice with DNA vaccines by the intramuscular route usually induced only a low or undetectable levels of neutralizing antibodies

Immunogen	Dose	One vaccination		Two vaccinations	
		Titer**	Survival	Titer**	Survival
pcJEME	100 ug x 2	<1:10	NT	1:10	5/5
pcJEME	10 ug x 2	<1:10	NT	1:10	5/5
PBS	x 2	<1:10	NT	<1:10	0/5
pcJEME	100 ug x 1	<1:10	4/5	NT	NT
pcJEME	10 ug x 1	<1:10	0/5	NT	NT
pcDNA3	100 ug x 1	<1:10	0/5	NT	NT

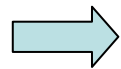
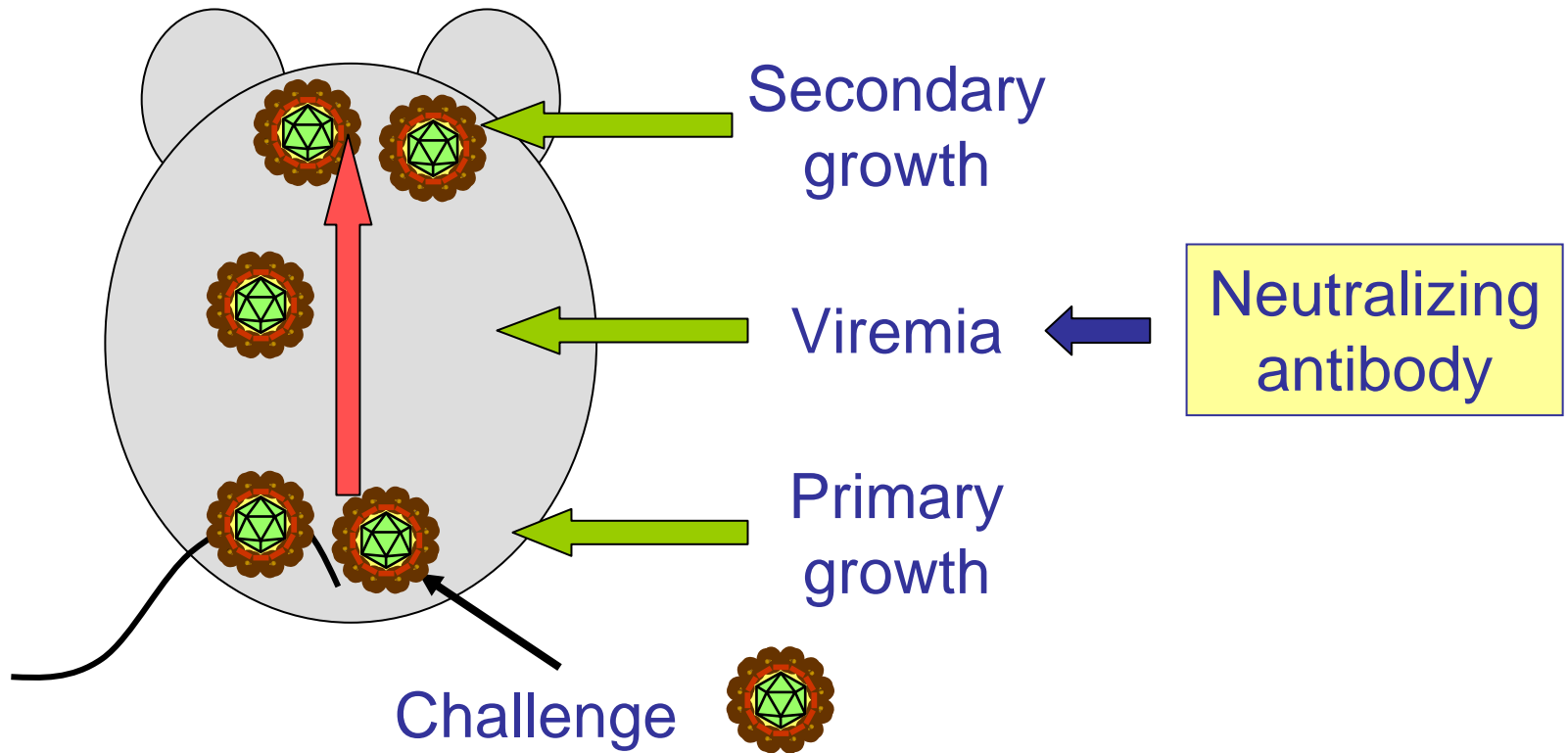


A model for protection by undetectable levels of neutralizing antibodies

# Anamnestic neutralizing antibody responses

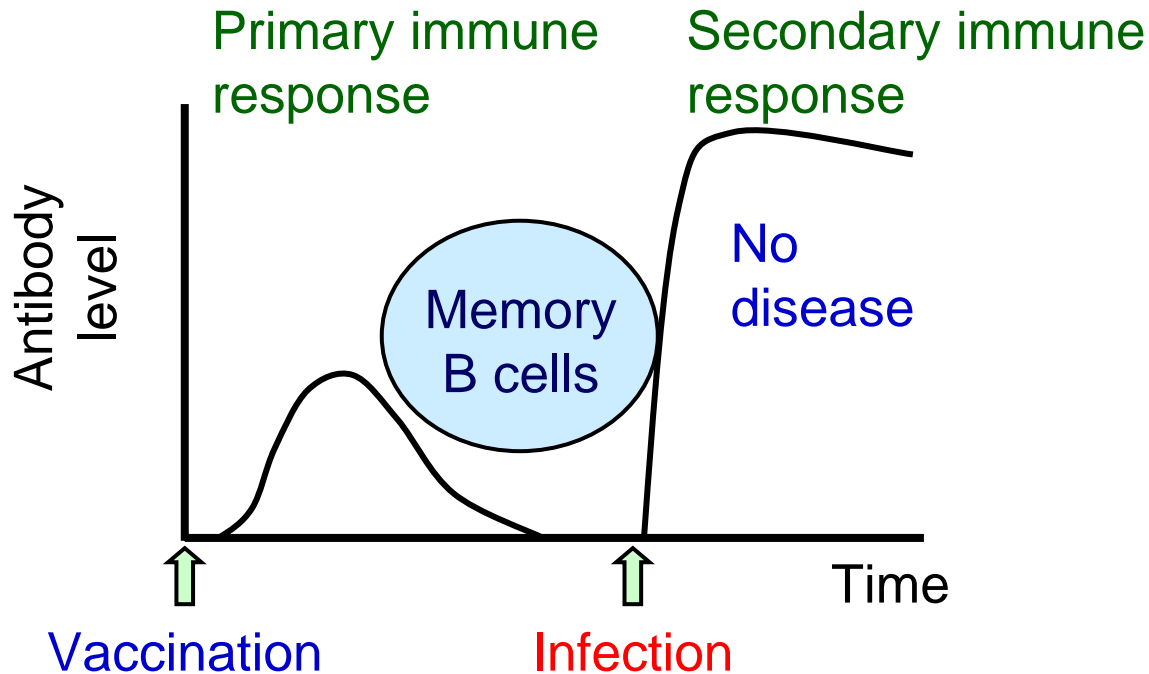


# Intrahost dissemination of JEV and a mechanism for protection



Neutralizing antibody is an effective factor that can block transportation of the virus from the peripheral replication sites to the brain

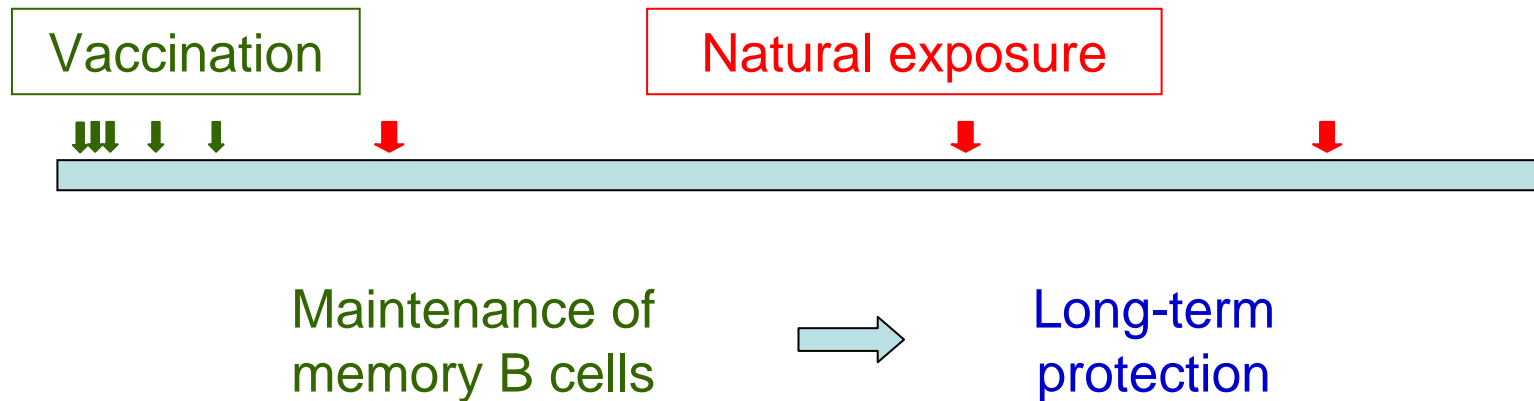
# Principle of vaccines



➡ Induction of memory B cells is an important role of JE vaccine.

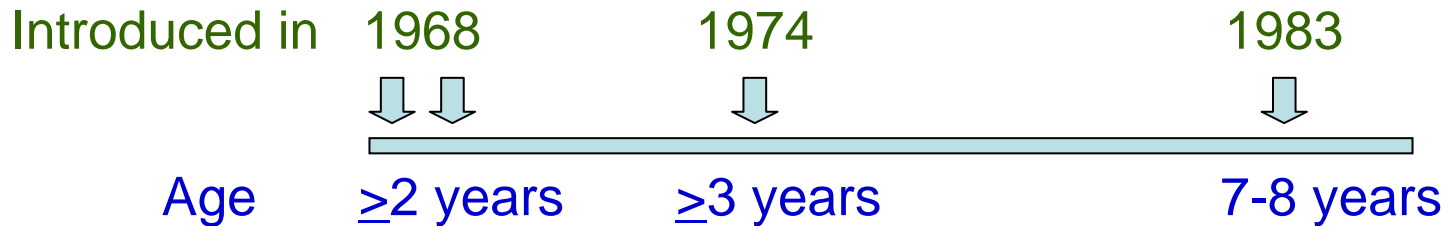
➡ Even though neutralizing antibodies are undetectably low at the time of infection, protection would be induced by the secondary immune responses.

Exposure to natural JEV infection may keep  
memory B cells for a long period



# A single dose is still effective

## JE vaccination program in Taiwan



Dose	Proportion of target population (%)	No. confirmed cases	Vaccine efficacy (%)
0	7.18	249	-
1	2.80	14	85.59
2	7.10	22	91.07
3	82.92	43	98.51

(modified from Yang et al., Vaccine 24, 2669-2673, 2006)

➡ Importance of the priming even by one dose

# SUMMARY

1. Small numbers of JE cases are maintained in Japan, although few people receive booster vaccinations.
2. The national JE surveillance program has shown natural JEV activities in swine.
3. Our surveys indicated that humans were still exposed to natural JEV infection.
4. Periodic exposure to natural infection with JEV may serve as natural “boosting” and maintain memory B cells in the vaccinated host for a long period.

# Collaborators

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